

## Copper Alloys for Human Infectious Disease Control

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### Abstract

Several bacteria, known to be human pathogens, die when placed on copper alloy surfaces. The concentration of live bacteria drops from several orders of magnitude to zero on copper alloys in a few hours. In marked contrast, no reduction is seen in the concentration of live organisms on stainless steel during the six-hour test period. The copper alloys tested include high coppers, brasses, bronzes, copper -nickels and copper-nickel-zincs. The bacteria tested include *E. coli* O157:H7 and *Listeria monocytogenes*, both food-borne pathogens associated with several large-scale food recalls, and Methicillin-Resistant *Staphylococcus aureus* (MRSA), a serious hospital-acquired infection. The study results suggest the selection of copper alloys for surfaces exposed to human touch or food contact. Using copper alloys in this manner can materially assist in reducing the transmission of potentially infectious organisms.

### Introduction

The utilization of copper alloys is continuous from ancient times to modern times: from an ornamental tin bronze frog cast in Mesopotamia in 3200 B.C. to the tin phosphor bronze connectors in your cell phone. Today copper alloys enjoy both mundane and esoteric applications: from copper plumbing tube and electrical wiring to high purity copper interconnects in microprocessors. The unique physical, mechanical and metallurgical properties, as well as other characteristics of copper alloys account for the enduring, diverse and growing uses of copper alloys. These include: high electrical and thermal conductivity, malleability, ductility, a range of mechanical properties and good corrosion resistance. In addition, copper alloys are easily alloyed, available in a range of aesthetically pleasing colors, highly recyclable, widely available, and, as will be presented in this paper, exhibit antimicrobial activity.

Throughout history, man has exploited the antimicrobial attributes of copper. An over 4000 year old Egyptian medical text, called Smith Papyrus, indicates that copper sterilizes drinking water and wounds. In 400 B.C., Hippocrates, the father of medicine, cited the use of copper for leg ulcers related to varicose veins. The ancient Aztecs used copper oxide and malachite, a copper carbonate compound, for treating skin conditions. Additionally, during the circa 1850 cholera epidemic in Paris, copper workers were found to be immune. In a recent laboratory study [1], pure cultures of a fecal indicator bacteria, *Escherichia coli*, were stored in a brass vessel of unidentified composition and an earthenware experimental control vessel for 6, 24 and 48 hours and then cultured. Results indicated that bacteria counts were reduced at 6 hours in the brass vessel versus the earthenware control. No bacteria were found in the brass vessel after 24 hours. As part of the same study, coliform bacteria counts were taken on brass and earthenware vessels in a village in Punjab, India, following overnight 12- to 15-hour storage. The initial counts at the source ranged from 1417 to 1773. A reduction in counts was seen in the brass vessel, down to a range of 53 to 131. The counts remained high in the earthenware vessel, at 1270 to 1677. In an earlier study [2], which was carried out in a hospital, brass doorknobs showed sparse growth of bacteria, while stainless steel doorknobs were found to be heavily contaminated. Results from a preliminary study [3] indicated that a commercial 99.95% copper alloy, (UNS C10200), and to a lesser extent Muntz metal (UNS C28000), a 60% copper-40% zinc-containing brass, inhibited the growth of *Escherichia coli* O157:H7, while no inhibition was observed on stainless steel (UNS S30400) surfaces.

The focus of the present study is on the inhibitory effects of the surfaces of a range of commercial wrought copper-base alloys, on bacteria, with stainless steel as an experimental control. The tested organisms include *E. coli* O157:H7 and *Listeria monocytogenes*, which are food-borne pathogens associated with several large-scale food recalls, and Methicillin-Resistant *Staphylococcus aureus* (MRSA), a serious hospital-acquired, or nosocomial infection. According to the March 28, 2001 issue

of the New York Times, 76 million illnesses associated with contaminated food were reported annually in the United States, which resulted in 325,000 hospitalizations and 5000 deaths. Although most *E. coli* strains are harmless to humans, the U.S. Dept. of Agriculture (USDA) estimates that the cost to society associated with infectious strains of *E. coli* is \$5 billion annually. The Centers for Disease Control (CDC) reported in 1999 that *L. monocytogenes* accounts for the highest hospitalization rate (90%) and the second highest fatality rate (20%) of all food-borne human pathogens. On average, there are 2,500 cases of *L. monocytogenes* are reported each year and they result in 500 fatalities. According to a July 2004 report by the Infectious Disease Society of America, two million people are infected each year while in the hospital, and 70% of those infections are resistant to at least one drug. This resulted in 90,000 deaths and a cost to society of \$5 billion annually. According to the CDC, the growth rate of antibiotic-resistant bacterial infection is increasing.

## Materials and Methods

**Table I: Nominal Alloy Composition (Weight %)**

Alloy UNS No.	Cu	Zn	Sn	Ni	Al	Mn	Fe	Cr	P	Si	Mg	Co
Copper												
C10200	99.95											
C11000	99.90											
C19700	98.95						0.7		0.25		0.10	
Brass												
C22000	90.0	10.0										
C23000	85.0	15.0										
C24000	80.0	20.0										
Y90*	78	12		3		7						
Bronze												
C51000	94.8		5.0						0.20			
C63800	95.0				2.8					1.8		0.40
C65500	97.0									3.0		
Cu-Ni												
C70600	88.6			10.0			1.4					
C71000	79.0			21.0								
Cu-Ni-Zn												
C75200	65.0	17.0		18.0								
C77000	55.0	27.0		18.0								
Stainless Steel												
S30400	0			8			74	18				
Plastic												
Polyethylene*	0											

\*no UNS number

The chemical compositions of the alloys utilized are listed in Table I. As can be seen, they range from coppers to brasses and bronzes, copper-nickels and copper-nickel-zinc alloys (nickel silvers). The other materials tested include polyethylene, from a household cutting board, and stainless steel (UNS S30400), which is widely used in food processing and healthcare applications.

As reported elsewhere [3-6], small, 1 cm x 1 cm coupons of each material were sheared for testing samples. *E. coli* O157:H7 was grown aerobically at 37°C in Tryptone Soya Broth (TSB) and stored on microbeads at minus 20°C. Microbeads were removed and thawed at room temperature in TSB and incubated at 37°C until used. For each experiment, cultures were between 15 hours and 20 hours old. A 20- $\mu$ l culture was placed on each coupon and allowed to air dry. After incubation, at 20°C or 4°C, the coupons were removed at predetermined times and transferred into 10-ml autoclaved, phosphate buffered saline (PBS), and the number of viable organisms remaining after various incubation times was determined using plate counts on nutrient agar (NA). The 20°C incubation time corresponds to room temperature, while the 4°C incubation time corresponds to refrigeration temperature. The tests were repeated an average of five times at 20°C and three times at 4°C. Furthermore, each data point at each temperature represents the mean bacterial count measured on several coupons. The number of coupons varied from alloy to alloy and also with temperature. The number ranged from two to six coupons, with four being the average. The methods were similar for *L. monocytogenes* [8] and MRSA [9], except that *L. monocytogenes* was cultured aerobically for 16 hours and 20 hours at 37°C using sterile Brain Heart Infusion Broth.

## Results

### *E. coli* O157:H7

A semi-log plot of time in minutes versus bacteria count for C11000, a 99.90% copper alloy, is shown in Figure 1. At 20°C, the bacteria count decreases by about one order of magnitude (one log) over 75 minutes and then falls off rapidly and reaches zero at 90 minutes. The zero point, which corresponds to a 9-log drop, indicates that the bacteria, which are *E. coli* O157:H7, are no longer viable and are dead. A similar pattern is seen at 4°C, but the times are longer, indicating that the rate of inactivation decreases as temperature decreases. A rapid falloff occurs between 180 minutes and 270 minutes at 4°C. On C10200, a 99.95% copper alloy, the tests were repeated four to six times at 20°C and three to four times at 4°C. Figure 1 represents data from a total of 71 coupons. A similar number of repeat tests and coupons was used to establish the plots shown in the other figures.

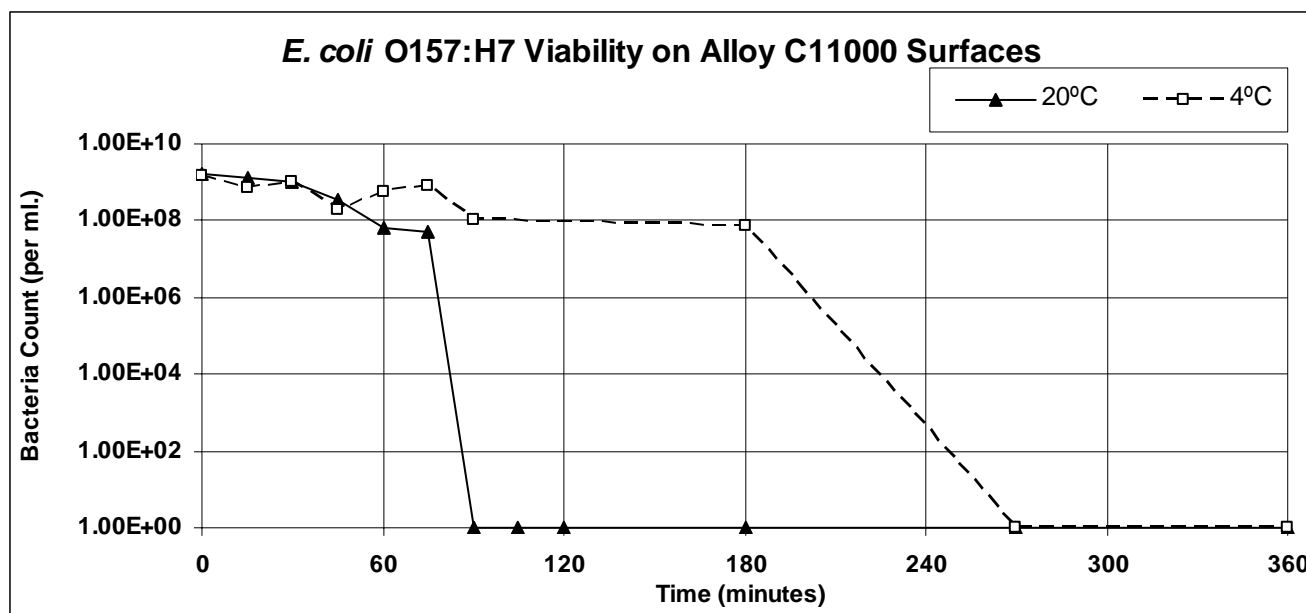


Figure 1: *E. coli* O157:H7 Viability on Alloy UNS C11000 Surfaces at 20°C and 4°C.

The bacteria count measured on alloy C23000, a brass containing 85% copper and 15% zinc, is shown in Figure 2. At 20°C, the bacteria count decreases slowly during the first 30 minutes. The count then shows a rapid 3-log drop at 45 minutes followed by another rapid 6-log reduction at 60 minutes where the bacteria count is zero. The total drop in bacteria count is 9 logs. Although no tests were run at 4°C for C23000, a similar pattern of longer times would be anticipated, based upon results seen in other brasses [4-6]. The inhibitory effects of the brasses on *E. coli* O157:H7 are similar to but lesser than those seen in the coppers. This is especially seen as the zinc content of the brasses reaches 20% and above, again based upon results seen in other brasses [4-6]. The brass alloys also showed a higher degree of tarnishing than all of the other alloys during these tests. Data are presented in Figure 2 for copper alloy Y90, which is the alloy on the surface of the Sacagawea U.S. one dollar

coin. Y90 is essentially a copper zinc alloy, or brass, with lesser amounts of manganese and nickel. The latter two elements improve tarnish resistance. Alloy Y90 follows the pattern seen brasses. At 20°C, the bacteria count decreases slowly, by one log, during the first 90 minutes. It then drops rapidly, by 8 logs, and reaches a zero bacteria count at 120 minutes. At 4°C, the bacteria count shows a 2-log decrease during the first 90 minutes, then a slight increase of one log over the next 90 minutes. At 180 minutes, it begins a rapid 7-log drop and reaches a zero bacteria count at 270 minutes.

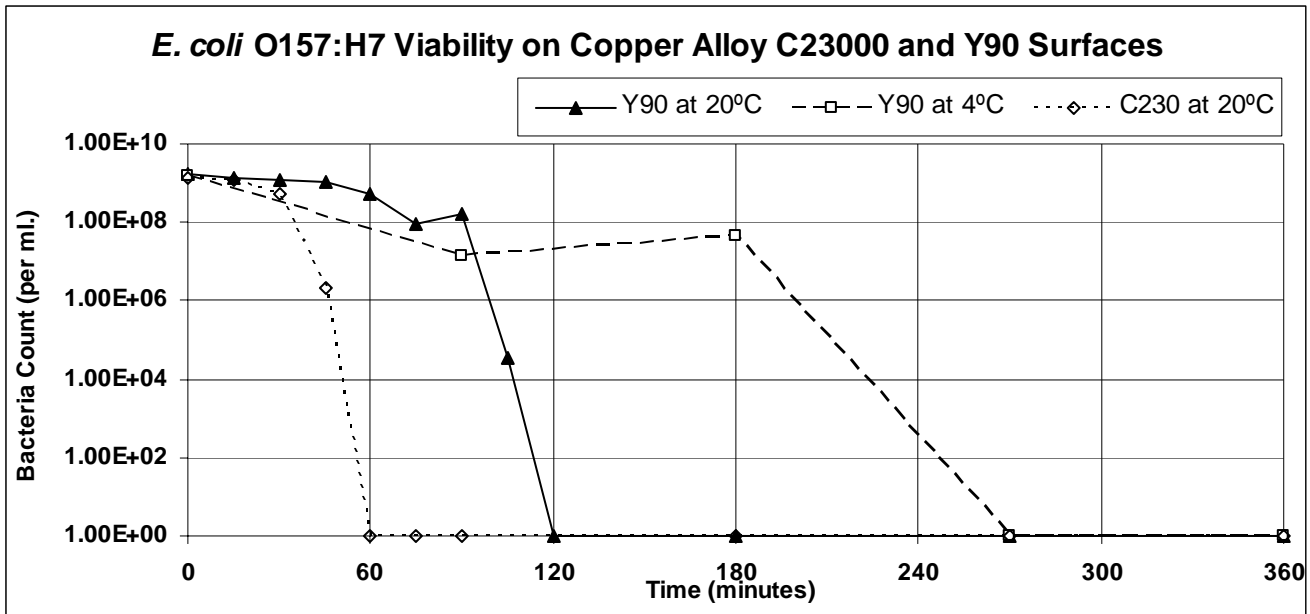


Figure 2: *E. coli* O157:H7 Viability on Alloy UNS C23000 at 20°C and Copper Alloy Y90 Surfaces at 20°C and 4°C.

Figure 3 shows the bacteria counts measured on C51000, a tin-phosphor bronze nominally containing 95% copper, 5% tin and 0.2% phosphorous, C71000, a copper-nickel containing 80% copper and 20% nickel, and C75200, an alloy containing 65% copper, 17% zinc and 18% nickel, which is called a nickel silver because of its color. On C51000, the bacteria count drops quite slowly, by about a half log, over the first 60 minutes and then falls off rapidly for the next 45 minutes and reaches zero at 105 minutes. The total drop in bacteria counts is again 9 logs. In C51000 at 4°C, the bacteria count falls slowly for the first 180 minutes, and then falls rapidly for the next 90 minutes, reaching zero at 270 minutes, as is also shown in Figure 3. Both C71000 and C75200 follow similar patterns. At 20°C, they both show very slow reductions during the first 90 minutes.

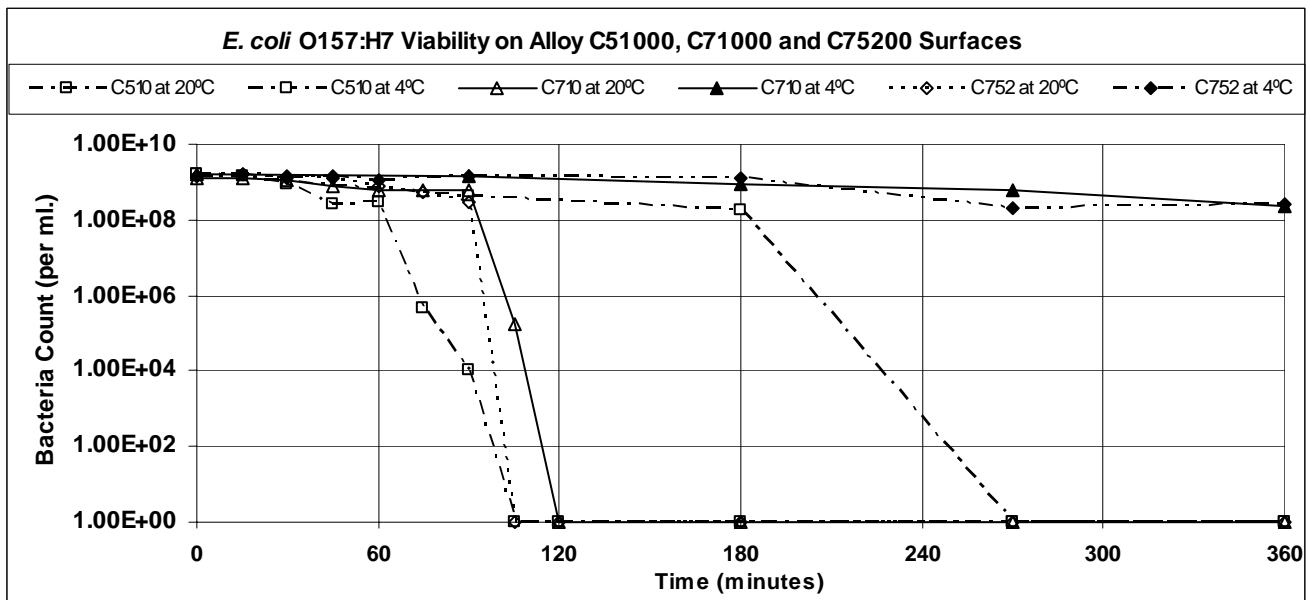


Figure 3: *E. coli* O157:H7 Viability on the Surfaces of Alloys UNS C51000, C71000 and C75200 at 20°C and 4°C.

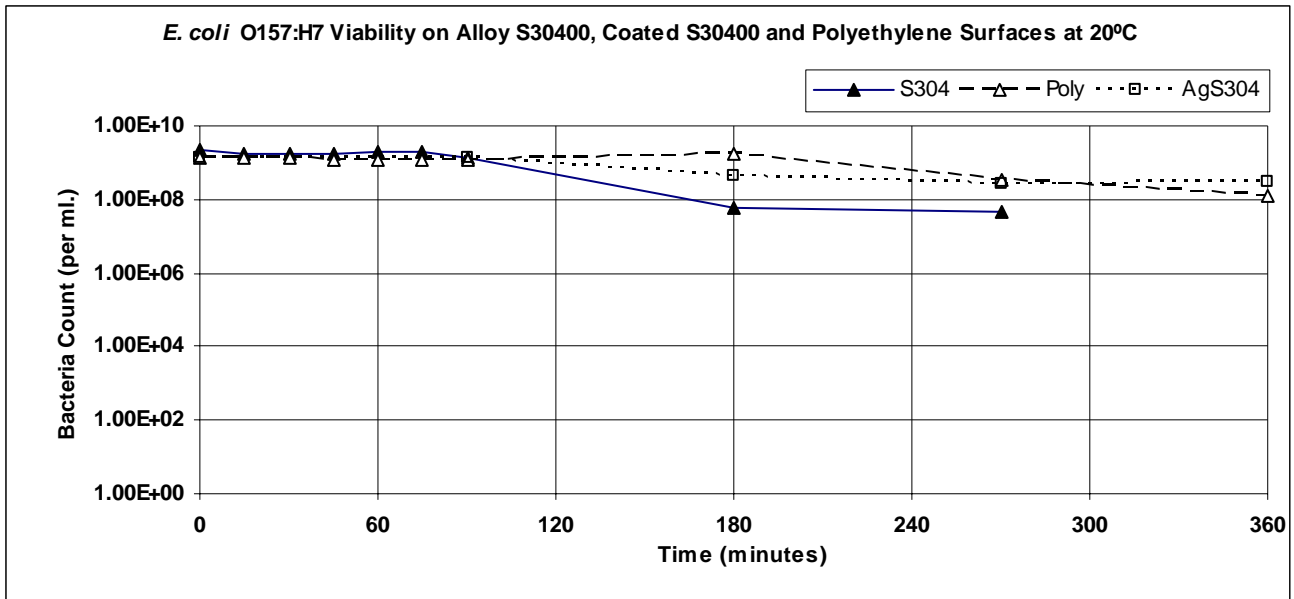


Figure 4: *E. coli* O157:H7 Viability on the Surfaces of UNS S30400, Polyethylene, and a Silver-containing Coating on UNS S30400 at 20°C.

Alloy C71000 then drops off rapidly during the next 30 minutes and reaches zero at 120 minutes, while C75200 takes only 15 minutes to fall off rapidly and reach zero at 105 minutes. At 4°C, both C71000 and C75200 exhibit a very slight decline of about a half log over the six-hour test period.

Three copper-free materials were also evaluated. These included stainless steel (S30400), which contains 74% iron, 18% chromium and 8% nickel and is widely used in food processing and healthcare applications, polyethylene, a common food cutting board material, and a stainless steel with a silver-containing coating. The latter is a commercial antimicrobial product. As can be seen in Figure 4, the bacteria count at 20°C is virtually unchanged on the uncoated stainless steel for the first 90 minutes and then falls by one log during the next 90 minutes. Although this test happened to be stopped at 270 minutes, results from a two-day test indicated that the uncoated stainless steel exhibited a 5-log drop at 20°C over two days, as shown in Figure 5, which also shows it exhibiting, at 4°C, a 2-log drop over two days.

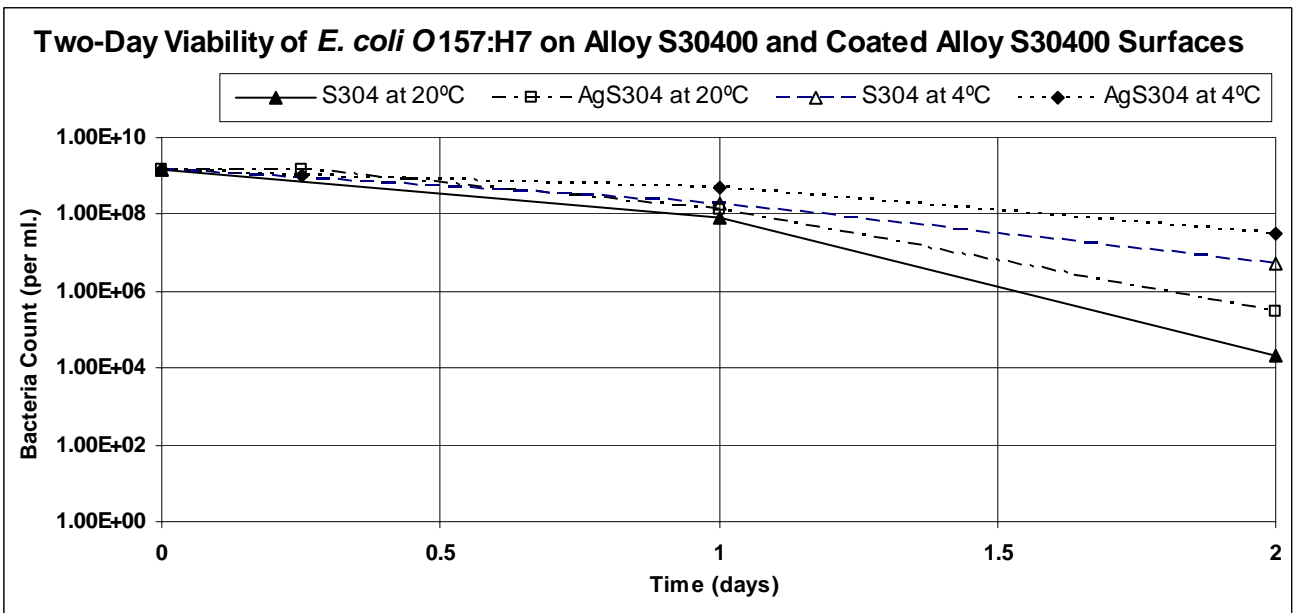


Figure 5: Two-day *E. coli* O157:H7 Viability on the Surfaces of Alloy UNS S30400 and a Silver-containing Coating on UNS S30400 at 20°C and 4°C.

During a long-term 28-day test at 20°C, uncoated stainless steel, again, exhibited a 5-log drop over two days, as shown in Figure 6. The bacteria count then remained constant at around 4 logs, for the next 26 days. Although this is a reduction of the bacteria count on stainless steel, it is known that only 10 to 50 organisms are sufficient to cause an infection in humans. During a long-term 28-day test at 4°C, stainless steel exhibited a 4-log drop over 7 days, as is also shown in Figure 6. The bacteria count then decreased slowly by one log, over the next 21 days. As shown in Figure 4, the bacteria count on polyethylene was unchanged for the first 180 minutes, and decreased by one log over the next 180 minutes. When compared to uncoated stainless steel, the silver-containing coating on stainless steel, which is a commercial antimicrobial product, displays were similar, but the latter exhibited somewhat less of a drop in bacteria count, as is also shown in Figure 5.

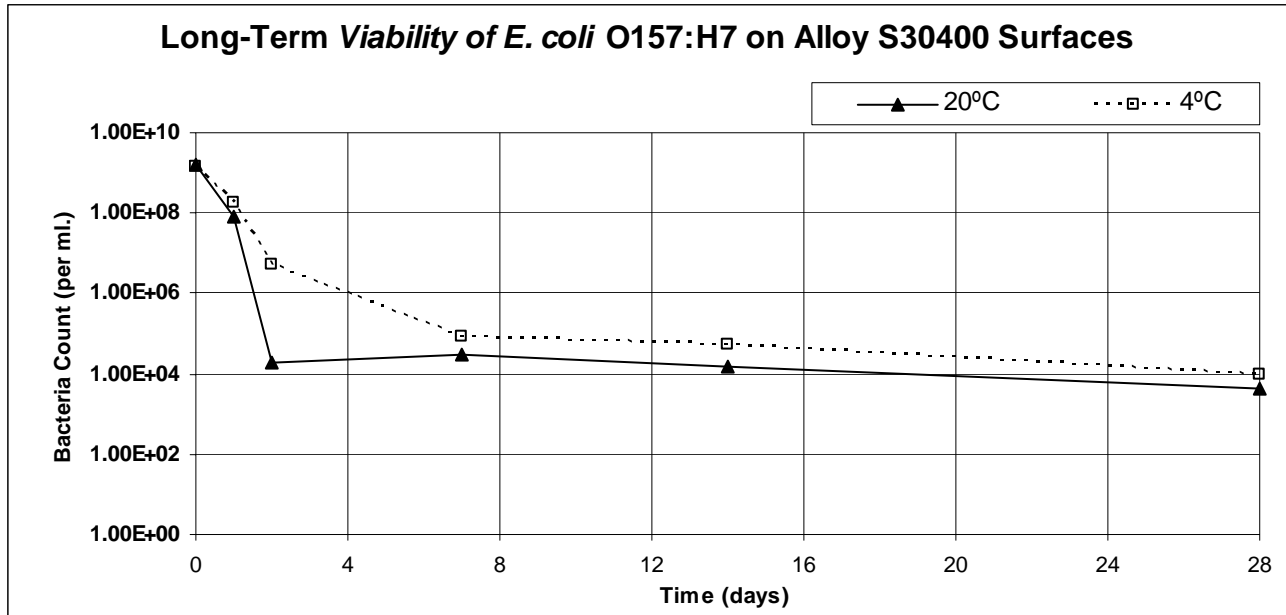


Figure 6 Long-term 28-day *E. coli* O157:H7 Viability on the Surfaces of Alloy UNS S30400 at 20°C and 4°C.

### Tarnishing

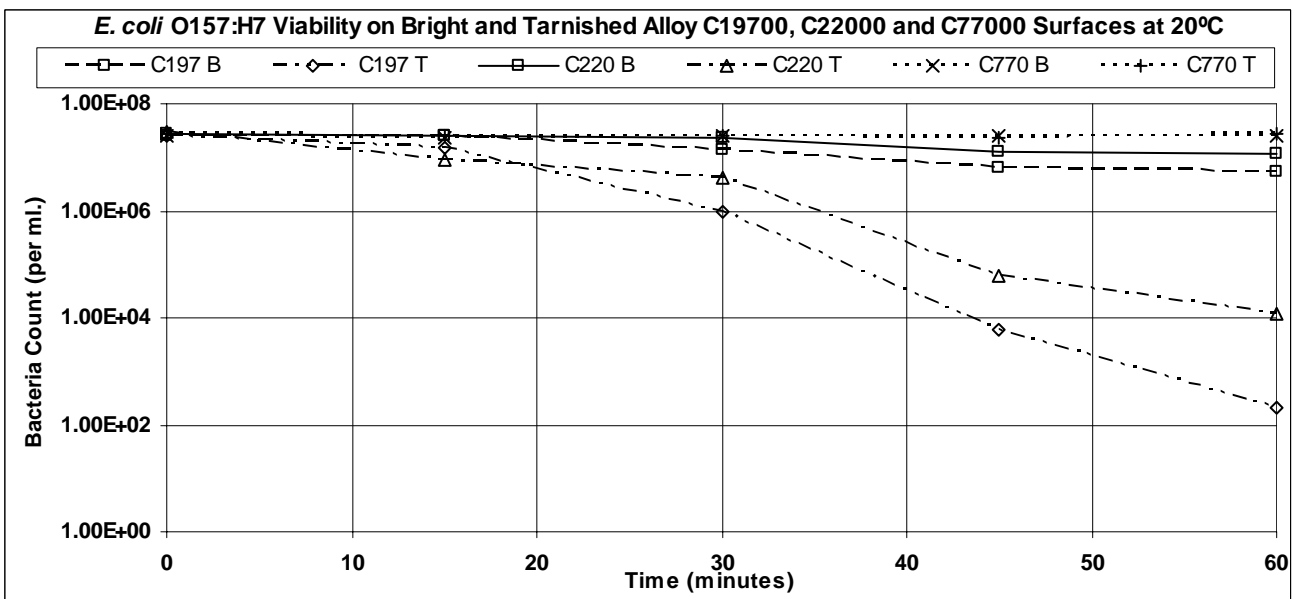


Figure 7: A Comparison of *E. coli* O157:H7 Viability on the Surfaces of Tarnished and Bright Alloys UNS C19700, C22000 and C77000 at 20°C.

The effect of tarnishing on the viability of three copper alloys was evaluated. Tests were run on three alloys which developed a brown tarnish film, which is probably cuprite ( $\text{Cu}_2\text{O}$ ), after a year of outdoor exposure in suburban New York City. *E. coli* O157:H7 counts were measured over a 60-minute test. The tarnish films were then removed with an abrasive cloth, and the alloys were retested in the bright condition, again, in a 60-minute test. The results for the three alloys, UNS C19700, an alloy containing 99% copper; C22000, a brass containing 90% copper and 10% zinc; and C77000, an alloy containing 55% copper, 27% zinc and 18% nickel, in both the tarnished and bright condition, are shown in Figure 7. Tarnished C19700 exhibited a 5-log drop over 60 minutes, as shown in Figure 7. When the tarnish was removed from each test coupon and the 60-minute test rerun, the results were much less dramatic and only a one-half log drop was recorded. In C22000, a similar but somewhat less of difference was seen. In the tarnished condition, C22000 dropped by 3 logs in 60 minutes, but when tested in the bright condition, this fell to less than one-half log. In C77000, no change in bacteria count was observed during the 60-minute test in either the tarnished or the bright condition. This was as expected, since no decrease in bacteria count was seen until 105 minutes in the previously reported 360-minute test of C77000 [5].

### Listeria monocytogenes

The viability of *L. monocytogenes* was measured on the surfaces of seven alloys [8]. The results at 20°C are presented in Figure 8. Bacteria counts were taken on C10200, an alloy containing 99.95% copper; C22000, a brass containing 90% copper and 10% zinc; C63800, an aluminum bronze containing 95% copper, 3% aluminum and 2% silicon; C65500 a silicon bronze containing 97% copper, 3% silicon and 1% manganese; C70600, a copper-nickel containing 90% copper and 10% nickel; C75200, an alloy containing 65% copper, 17% zinc and 18% nickel; and S30400, a stainless steel containing 74% iron, 18% chromium and 8% nickel. Four alloys, C10200, C22000, C63800 and C65500, which all contain 90% or a greater amount of copper, follow the same pattern, as shown in Figure 8. The bacteria counts drop slowly, by about 1.5 to 2 logs, over the first 45 minutes and then fall rapidly over the next 15 minutes and reach zero at 60 minutes. A total reduction of 9 logs is seen in bacteria counts. C70600 follows a similar but somewhat delayed pattern, as is also shown in Figure 8. The bacteria count drops slowly over the first 60 minutes, by about a half log, and then falls rapidly over the next 30 minutes and reaches zero at 90 minutes. In marked contrast, the stainless steel exhibits no change in its bacteria count for the duration of the test, which was 90 minutes.

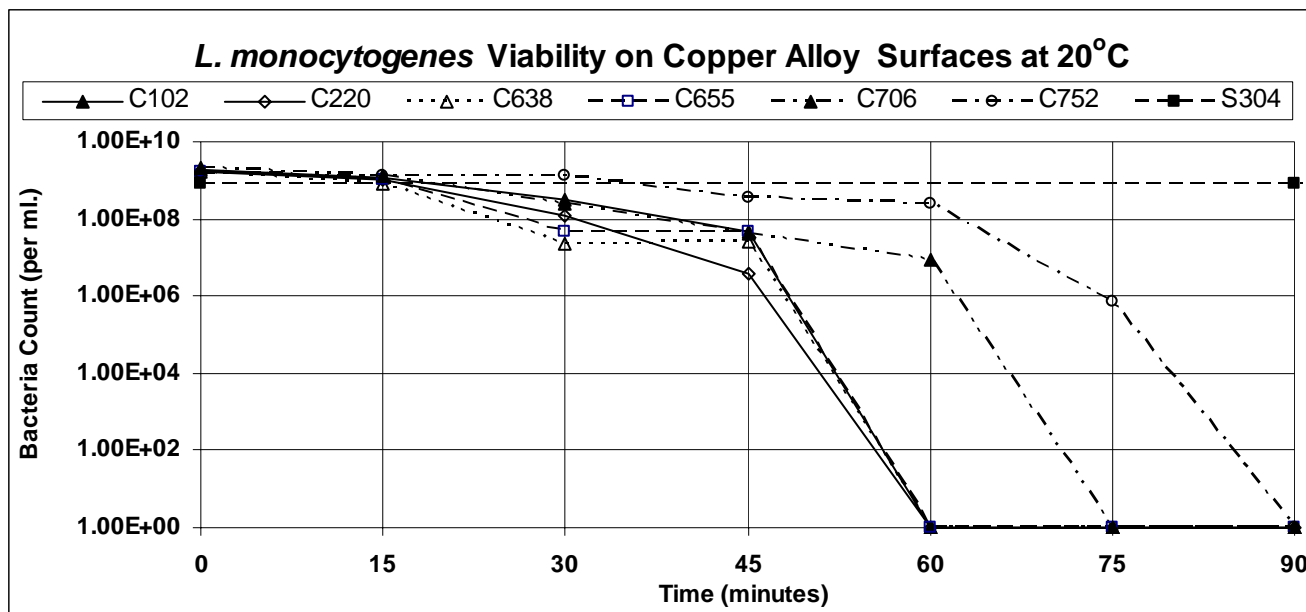


Figure 8: The Viability of *Listeria monocytogenes* on the Surfaces of Alloys UNS C10200, C22000, C63800, C70600, C75200 and S30400 at 20°C.

### Methicillin-Resistant Staphylococcus aureus

The viability of Methicillin-Resistant *Staphylococcus aureus* (MRSA) was measured on the surfaces of four alloys [9]. The results at 20°C are presented in Figure 9. The bacteria, counts taken on C19700, an alloy containing 99% copper; C24000, a

brass containing 80% copper and 20% zinc; C77000, an alloy containing 55% copper, 27% zinc and 18% nickel; and S30400, a stainless steel containing 74% iron, 18% chromium and 8% nickel. On C19700, a rapid 7-log falloff to zero is seen within 75 minutes, while on C22000 a uniform 7-log drop to zero occurs in 270 minutes, as shown in Figure 9. In C77000, 3-log drop is observed after 270 minutes. That may not seem very dramatic in comparison to the 7-log drop seen in C19700 and C22000, but that is a 99.9% reduction in live bacteria.

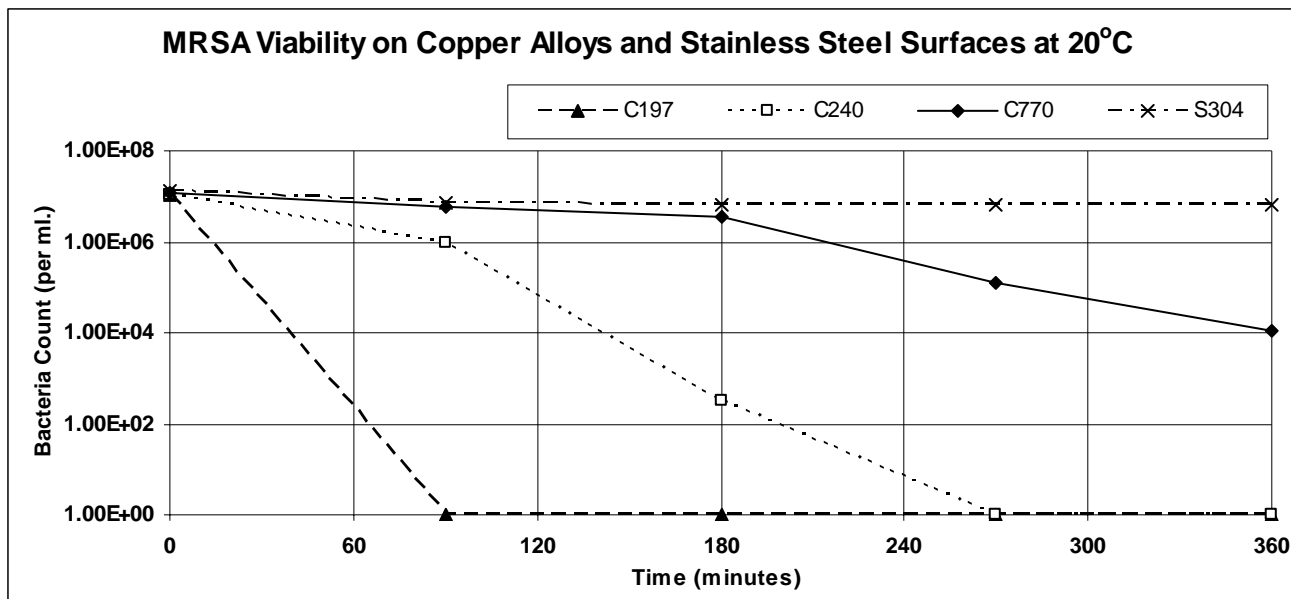


Figure 9: The Viability of Methicillin-Resistant *Staphylococcus aureus* (MRSA) on the Surfaces of Alloy UNS C19700, C24000, C77000 and S30400 at 20°C.

## Discussion

Several trends are apparent from this study. The first is that the inhibitory effects of a given copper alloy decreases as temperatures decreased, from 20°C to 4°C. This can be seen in Figures 1 through 3 for *E. coli* O157:H7 on C11000, Y90, C51000, C71000 and C75200 surfaces. The second trend is that the inhibitory effects decrease as copper content of the alloys decreases. This can be seen in Figure 8 for *L. monocytogenes* and in Figure 9 for MRSA. In Figure 8, the bacteria count of *L. monocytogenes* on the first four alloys, C10200, C22000, C63800 and C65500, which range in copper content from 90% to 99.9%, falls to a zero within 60 minutes at 20°C. Another 90% copper alloy, C70600, reaches the same point at 75 minutes, while it takes 90 minutes for the 65% copper alloy, C75200, to reach a zero bacteria count. The slower response observed in C75200 is probably related to its lower copper content. However, the slower response, or longer time to drop to zero bacteria count observed in C70600, at 75 minutes, may be a result of the better corrosion resistance of C70600 relative to these first four alloys. Better corrosion resistance correlates with lower availability of the cupric ion, Cu<sup>+2</sup>. The cupric ion is believed to be responsible for the antimicrobial action of copper.

Data for the time to reach a zero count of *E. coli* O157:H7 was extracted from two previous publications [5, 6], and a report [10]. Although a total of 25 copper alloys were tested, two of those alloys that did not induce a zero bacteria count during the duration of the 360-minute test were not plotted. At 20°C, C77000, the alloy with lowest copper content (55%) that was evaluated, and C71500, a very corrosion resistant 70% copper – 30% nickel alloy, did not reach a zero bacteria count in 360 minutes. At 4°C, nine alloys, which had tended to have either lower copper content and/or better corrosion resistances, did not reach zero bacteria count in 360 minutes. The copper content of the specific alloy versus the time to reach a bacteria count of zero was plotted for the remaining alloys at 20°C and at 4°C. A computer program was utilized to calculate and establish a linear trend line for each of the two temperatures, as shown in Figure 10. The scatter in data points is most likely related to the variation in the corrosion resistance of this broad range of copper alloys. One would expect variations in the corrosion resistance of various copper alloys at a given copper content. For example, at 70% Cu, UNS C26000, a brass with 30% zinc, would typically have lower corrosion resistance than UNS C71500, 70% Cu and 30% nickel. The greater corrosion resistance of the nickel-containing alloy correlates with the lower availability of the cupric ion, Cu<sup>+2</sup>.



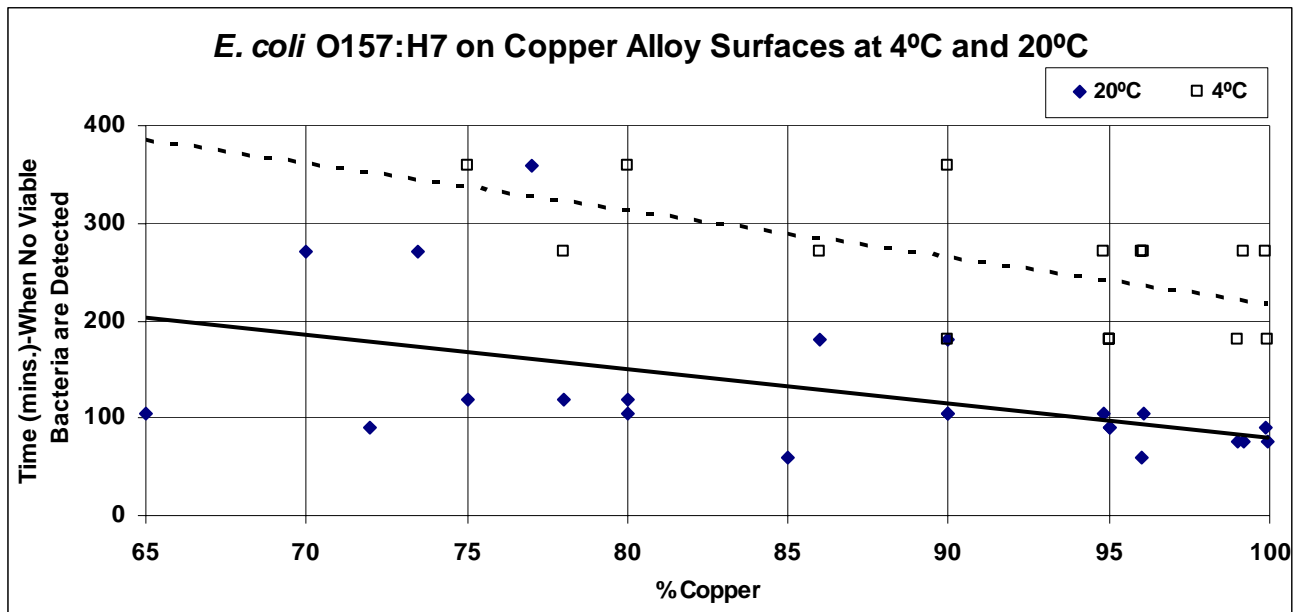


Figure 10: Time at which No Viable *E. coli* O157:H7 are Detected at 20°C and 4°C on the Surfaces of 23 Copper Alloys.

In contrast to the copper alloys, the stainless steel, UNS S30400, a popular material for food-processing equipment and healthcare applications, has little or no inhibitory effect. This is shown in Figures 4, 7 and 8 for *E. coli* O157:H7, *L. monocytogenes*, and MRSA. During the *E. coli* O157:H7 test, UNS S30400 shows a 5-log drop in bacteria count, as shown in Figure 5. It remains at that level, slightly above 5 logs, for the balance of a 28-day test, as shown in Figure 6. Similarly, in two days, the antimicrobial silver-containing coating on stainless steel shows a 4-log drop to a little above 5 logs, which is one log higher than seen on uncoated stainless steel. This is still a high bacteria count for both materials, especially when ingestion of only 10 to 50 individual bacteria may be sufficient to cause infection. Thus, stainless steel surfaces and, particularly, those with a silver-containing coating still have a potential to adversely affect human health after two days. These levels persisted for 28 days in the case of uncoated UNS S30400, at which point the test was terminated.

Alloys C19700 and C22000 in the tarnished condition exhibited an antimicrobial effect at shorter times than their bright counterparts. The  $\text{Cu}_2\text{O}$  tarnish film may more readily release  $\text{Cu}^{+2}$  than a bright metal surface.

The ways in which copper acts on microorganisms is a complicated subject, and beyond the scope of this paper. However, a few of the many proposed mechanisms include:

- If proteins are complexed or altered, they can no longer perform their normal functions [11].
- Copper complexes form radicals (that inactivate viruses) [12, 13].
- Metals, such as copper, may disrupt enzyme structure [14].
- Inactivation is due to oxidation potential of the ion [15].
- Transition metals (including copper) facilitate deleterious activity in superoxide radicals [16].
- $\text{Cu}^{+2}$  can form protein chelates through a protein's carboxylate and amino groups, thereby inactivating a protein [17].
- Divalent cations, such as  $\text{Cu}^{+2}$ , may strain protein structure, causing hydrogen bonds within the DNA to break, and thus opening the double helix [11].
- If the nucleic acid helix is stabilized or destabilized by chelation with a metal ion, replication or transcription is altered, thereby rendering the microorganism inactive [11].
- $\text{Cu}^{+2}$  has a specific affinity for DNA and can bind and disorder helical structures by crosslinking within and between DNA strands [18].
- $\text{Cu}^{+2}$  may complex messenger RNA (and thereby play a role in disinfection of viruses) [19].
- $\text{Cu}^{+2}$  has a strong affinity for  $\text{O}^-$ ,  $\text{N}^-$  and  $\text{S}^-$  sites in proteins [20].

- Disinfection due to metal complexes is important in enabling a possible entry through a cells membrane [11].
- Copper can interact with lipids, causing their peroxidation and opening holes in the cell membrane [21].
- Studies on copper-injured *E. coli* cells indicate that the respiratory chain is damaged at least one site [22] and is associated with impaired cellular metabolism [23].

Thus, it appears that Cu<sup>+2</sup> has the potential to disrupt cell function in several ways. Since several of these mechanisms may be acting simultaneously, this may reduce the ability of the microorganisms to develop resistance to copper. In summary, the above mechanisms provide insight related to the complicated and incompletely understood antimicrobial action of copper.

Any human health claim for a nonfood contact surface is regulated by the U.S. Environmental Protection Agency (EPA). In particular, it falls under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). At present, EPA registration for copper alloys, under FIFRA, is being pursued. Until this EPA registration is secured, marketing of copper alloys as antimicrobial materials to protect human health is not permitted. Copper alloys can play an important role in the control of harmful infections. However, it must be noted that the use of copper alloys will not be advocated as a substitute for good housekeeping and hygienic practices, only as a supplement. Registration for food processing and other applications will also be pursued.

Future work will evaluate the inhibitory effects of copper alloys on other human pathogens, including other types of bacteria, molds that contribute to respiratory distress (a type of infection that is associated with “Sick Building Syndrome”) and viruses. One mold, *Aspergillus niger* and one virus, Influenza A, have been tested. Preliminary results indicate that copper alloys also inhibit their viability.

Now that the effects of a range of copper alloys have been demonstrated on the viability of a variety of bacteria and at two temperatures, efforts will focus on the development of additional information needed for the adoption of copper alloys by healthcare and food-processing equipment manufacturers. This will include tests on durability, cleanability, the effects of common disinfectants and corrosion resistance in environments that directly relate to the intended application, healthcare and food processing equipment and food preparation surfaces.

The utilization of copper alloys for door handles, door push plates, faucets, bedrails, stair and corridor rails and other hardware, holds the promise of being an effective, passive approach to controlling MRSA in healthcare facilities, including hospitals, clinics, physician’s examination rooms and nursing homes. Other target markets include schools, public buildings, shopping malls, hotels, gyms, prisons, mass transit systems, airports and cruise ships.

In summary, the results suggest that copper alloys that have antimicrobial properties can be used for surfaces exposed to human touch or contact with food and can contribute to a reduction in the transmission of potentially infectious human pathogens.

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